

## **EXHIBIT D**



## Development and registration of recombinant veterinary vaccines The example of the canarypox vector platform

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### Abstract

The canarypox vaccine vector (ALVAC<sup>®</sup>) technology has been used to develop and license several vaccines for companion animals and horses in the European Union and USA. ALVAC is a ubiquitous vector with high biosafety since it is non-replicative in mammals, is genetically and physically stable, and able to induce both humoral and cell-mediated immune responses against the expressed transgene product. Specific rules apply for the development and registration of recombinant vector vaccines. The biology of the vector as well as the recombinant virus must be thoroughly documented to allow the risk assessment of its use in the target species. In particular, its safety for the host and the environment must be extensively demonstrated before field trials can be authorized.  
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### 1. Introduction

Veterinary medicine has been at the forefront of cutting edge vaccine technology as illustrated by the number of non-conventional vaccines available in the animal health market. Recombinant protein subunit vaccines against Lyme disease in dogs [1], feline leukemia [2] or classical swine fever [3] have been developed by using either *Escherichia coli* or baculovirus expression platforms. Gene-deleted porcine and bovine viral vaccines have been instrumental in the prophylaxis and local eradication of Aujeszky's disease and infectious bovine rhinotracheitis [4]. Very recently, two DNA vaccines have been licensed for immunization of horses against West-Nile virus (WNV) in the USA and for immunization of fish against infectious hematopoietic necrosis in

Canada [5]. Interestingly, live recombinant vector vaccines for food animals, as well as for companion animals represent the majority of those new technology vaccines, and most of them are based on poxvirus vectors.

The use of poxvirus vector started with the development of RABORAL V-RG<sup>®2</sup> (Merial, USA) a rabies vaccine for wildlife [6]. Oral immunization of red foxes, raccoons and coyotes was made possible by using the vaccinia Copenhagen thymidine kinase-negative strain to express rabies glycoprotein G [7]. This vaccine has successfully contributed to the eradication of sylvatic rabies from some countries of Western Europe and is now widely used in North America for the control of rabies in raccoons and coyotes [8].

Avipoxviruses and in particular the fowlpox virus vector were initially developed for poultry vaccines to protect against Newcastle disease and avian influenza H5 [9]. The fowlpox virus vector was derived from an attenuated vaccine

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<sup>1</sup> ©: ALVAC is a registered trademark of Connaught Technology Corporation in the USA.

<sup>2</sup> : RABORAL VR-G is a registered trademark of Merial in the USA and elsewhere.

Table 1  
Canarypox virus vector vaccines licensed for veterinary use

Target	Inserts	Species	Country
Canine distemper	HA and F	Dog, ferret	USA, Argentina, Brazil, Colombia, Canada, Uruguay
Rabies	G	Cat	USA, Canada
Feline leukemia	Env, Gag/pro	Cat	Europe, USA, Canada
Equine influenza	HA	Horse	Europe
West-Nile	prM-E	Horse	USA, Canada

HA, hemagglutinin; F, fusion protein; G, glycoprotein G; Env, envelope glycoprotein; Gag, group specific antigen; Pro, protease; prM-E, pre-membrane and envelope proteins.

strain with a long record of safe use in chickens. Although it is a replicative vector in chickens, it is not shed in the environment after vaccination. Unfortunately, anti-fowlpox virus active immunity interferes with the vector efficacy and may limit its use in some field conditions.

In 1988, the ability of avipoxvirus to express a transgene in a mammalian host and induce a specific immune response in the absence of replication was demonstrated with an experimental fowlpox-Rabies construct [10]. The development of avipoxvirus vectors for mammals was extremely attractive from a biosafety standpoint. Efficacy results against Rabies suggested that the canarypox virus was a better candidate than the fowlpox virus for the development of a non-replicative vector for mammals [11]. Several canarypox virus recombinant vaccines have been developed and licensed for dogs, cats, ferrets and horses (Table 1), and many new candidates are currently being tested in both animals and humans.

Even if non-replicative vectors are attractive, replication competent vectors are widely studied and may be safely used. Attenuated vaccine strains may be selected as vectors, like LaSota attenuated strain of Newcastle disease virus in poultry [12] or canine adenovirus type 2 in dogs [13]. Recently, a vaccine against infectious bursal diseases using the herpesvirus of turkey (HVT) was licensed for in ovo vaccination and immunization of 1-day-old chicks [14]. From a biosafety standpoint, HVT is not pathogenic for turkeys or chickens, and is not spread from chicken to chicken after vaccination. A West-Nile virus chimera vaccine has been developed for horses by inserting the prM-E gene of WNV at the site of the E protein of a Yellow Fever virus attenuated strain [15]. In the research and development of human vaccines, many viral virus vectors, replication competent or not, are under investigations. However, vectored vaccines tested in clinical trials are generally based on vectors which do not replicate in humans, like the modified vaccinia virus Ankara strain [16], replication-defective adenovirus [17], alphavirus replicons [18] and avipoxviruses [16] including canarypox virus [19–25].

## 2. Advantages of the canarypox virus vector

The ALVAC vector is a canarypox virus clone obtained after four rounds of plaque purification of a strain from a vaccine for canaries. Despite an abortive replication cycle

in mammalian cells, ALVAC can express appropriately engineered foreign genes resulting in de novo synthesis of proteins which are then presented to the immune system in a manner similar to a natural infection and generate both antibodies (Ab) and cell-mediated immunity (CMI).

### 2.1. Efficacy

Veterinary vaccines are required to meet protection standards in the target species regardless of the type of immune response they induce. Nevertheless, the ability of the canarypox vector to induce both Ab and CMI against specific antigens has been evaluated in several models and species [26,27].

A recombinant canarypox vaccine expressing the envelope glycoprotein (*env*), the capsid proteins (*gag*) and part of the polymerase (*pro*) genes of the feline leukemia virus (FeLV) subgroup A, has been shown to protect cats in various FeLV challenge models (oro-nasal or intra-peritoneal challenge, contact challenge) [28–30]. Prevention of persistent FeLV p27 antigenaemia relies on a FeLV specific T cell response which can be measured as early as 1 week post-infection, while it takes several weeks before FeLV neutralizing antibodies can be detected. *Env* and *gag* are the main targets of cytotoxic T cells [31]. In cats, ALVAC-FeLV provides protection in the absence of FeLV neutralizing antibodies at the time of virulent challenge. It has recently been shown that ALVAC-FeLV vaccinated cats had FeLV-specific IFN $\gamma$ <sup>+</sup> T cells detectable before virulent challenge, supporting a predominant role of CMI in the protective efficacy of this vaccine [32]. The performance of the ALVAC-FeLV construct is achieved in the absence of adjuvant, ensuring an excellent local safety and minimum inflammatory reactions. This advantage, already demonstrated for the ALVAC-Rabies vaccine, is now substantial in feline vaccinology, as chronic inflammation at the injection site is considered to be a risk factor for the occurrence of injection site associated fibrosarcomas [33].

The capacity of some ALVAC-based vaccines to induce protection after a single dose is an additional advantage when a rapid onset of immunity is required. A single dose of recombinant canarypox virus, expressing the prM-E polyprotein of a 1999 New York isolate of West-Nile Virus, was proven effective in horses against a WNV-infected mosquito challenge, a method representative of the natural exposure in the

field [34]. Following challenge, only 1 of 9 treated horses developed viremia, whereas 8 of 10 control horses became viremic. Although this vaccine has been licensed with a two-dose regimen, this study demonstrated the rapid onset of immunity associated with the ALVAC vector. Protection after a single vaccine dose was also observed in horses with another canarypox vector expressing the haemagglutinin genes of influenza H3N8 viruses [35].

Maternal antibodies is a concern for the efficacy of vaccination in young animals, and in some models, a vectored vaccine may allow an early and efficacious vaccination. A recombinant canarypox construct expressing the HA and F genes of canine distemper virus (CDV) [36] was developed specifically for dogs as a safe and efficacious alternative to the live CDV vaccines. Indeed, some live attenuated strains are either over- or under-attenuated and have been associated, respectively, with rare episodes of reversion to virulence, or lack of immune responses in part of the vaccinated dog population. In this model, a significant advantage of ALVAC-CDV is its capacity to overcome CDV maternal antibodies in young puppies. Four-week-old pups with maternal antibodies were vaccinated twice at a 4-week interval either with a canarypox-distemper or a conventional attenuated vaccine. Ninety percent of ALVAC-CDV immunized animals seroconverted while only 50% of pups vaccinated with conventional attenuated vaccines did [37].

One concern with some viral vectors is the interference of anti-vector immunity on the efficacy of subsequent revaccination. Immunization programs in companion animals and horses require several boosters at various intervals depending on the vaccine. ALVAC is a vector of choice in that context because anti-canarypox virus immunity does not alter the performance of boosters. Previous exposure to the vector does not inhibit humoral and cell-mediated responses against the transgene after revaccination, as experimentally demonstrated with the ALVAC-WNV vaccine (El Garch et al., submitted). The absence of detectable neutralizing antibodies against ALVAC, even after administration of repeated high doses, may be an explanation for this phenomenon. This advantage is also true for combination vaccines containing several canarypox virus recombinants. In addition, recombinant canarypox virus vaccines can be used to boost the immune response induced by conventional vaccines, as demonstrated for ALVAC-WNV in horses or ALVAC-FeLV in cats [38,39].

Considering the non-replicative nature of canarypox vector, it was important to evaluate the duration of immunity (DOI) induced by ALVAC-based vaccines. A non-adjuvanted canarypox-FeLV vaccine was shown to protect cats against persistent viraemia following a FeLV oro-nasal challenge carried-out 1 year after vaccination [28]. Similarly, horses immunized with ALVAC-WNV were protected against clinical signs and viraemia induced by a mosquito WNV challenge 1 year after vaccination [40]. Licensure of veterinary vaccines in Europe requires the demonstration of DOI by vaccination and viral challenge, and the dose of canarypox virus used in

the DOI studies determine the claimed minimum protective dose of the vaccine.

## 2.2. Safety

As for conventional attenuated vaccines, safety must be evaluated in the target species under laboratory and field conditions. In Europe, laboratory studies include the safety of an overdose, which is equal to 10 times the maximum release dose of the vaccine, and the safety of the repeated administration of one dose. These studies are done in the most susceptible animals, such as animals at the minimum age recommended for vaccination. The safety of ALVAC-based vaccines has been extensively evaluated in cats, dogs, ferrets or horses. At very high doses administered subcutaneously (up to  $10^{9.4}$  CCID<sub>50</sub>/dose), the most common post-vaccinal reactions consist of transient hyperthermia with or without lethargy, mild and transient local pain and swelling at the injection site. This reactogenicity has been a consistent feature of the various ALVAC constructs tested so far. Histological examination showed that the inflammatory reaction induced by ALVAC-FeLV at the injection site was mild, localized and transient (Day et al., submitted). At commercial dose, the reactogenicity of recombinant canarypox virus vaccines is low: in large scale field safety trials with combined canarypox-FeLV vaccines in cats, the rate of local reactions, such as pain, itching and transient swelling ranged from 1 to 3.6%. A similar safety profile has been reported in humans [41].

Conventional distemper MLV vaccines may be fatal to grey foxes, red pandas, black-footed ferrets, kinkajous and African Cape hunting dogs [42]. Interestingly, some exotic mammals (red pandas, giant pandas, black footed ferrets, cheetahs, tigers, pumas, leopards and Santa Catalina Island foxes) have been safely vaccinated with ALVAC-distemper without any risk of post-vaccine distemper disease.

More generally, safety has been confirmed in a wide range of species as well as by different routes of administration (Table 2).

## 2.3. Practical aspects

Vaccination of companion animals and horses has been done with combination vaccines containing live and killed antigens, sometimes combined with an adjuvant. The ability of ALVAC-based vaccines to be incorporated in existing combination vaccine formulations has been an important factor in the success of this vector. For example, ALVAC-FeLV does not alter the efficacy of the other feline vaccines, as demonstrated by serology and virulent challenge. Conversely, the other feline antigens do not interfere with the efficacy of the canarypox-FeLV, as illustrated by the similar performances of a monovalent and a combined ALVAC-FeLV in a duration of immunity study [28]. Generally, compatibility of ALVAC-based vaccines has been demonstrated with various inactivated or live vaccines and with adjuvants like polymers

Table 2

Safety of ALVAC-based vaccines has been demonstrated in various species and conditions of use

Species	Cat [29], dog [36], horse [34,35], ferret [54], sheep [50], pig [51], monkey [26,44], rabbits, guinea-pigs, mice [44], Canary [44], chicken, duck, goose, crow [40], human [19–25,55]
Age	Young animals, new-born mice [46]
Immune status	Nude mice, cyclophosphamide treated mice [55], FIV or FeLV infected cats [29], immuno-compromised individuals: HIV infected humans [21], cancer patients [22–25]
Route of administration	Subcutaneous [29,44], intra-muscular [34,35], transdermal [32,44,55], oral [44,55], ocular [44], intra-venous [56], intra-cerebral [44], intra-tumoral [57]

(carbomer) or oil-in-water emulsions. In addition, the physical stability of the canarypox virus allows the formulation of either freeze-dried or liquid vaccines with shelf-lives up to 2 years.

### 3. Development and registration of live recombinant vector vaccines for veterinary use

The development of a live recombinant vector vaccine is highly regulated. It follows the general rules of the development of conventional attenuated vaccines. In addition, a very detailed characterization of the vaccine strain must be provided, as well as an extensive evaluation of its biosafety, supported by specific studies.

Pari II.H of the European registration file and the Summary of Information Format (SIF) document must be submitted to US authorities in order to obtain authorization to conduct field trials. These documents contain a detailed description of the genetically modified organism and the risk assessment of its release into the environment. This description includes information on the vector itself, the inserted genes and the properties of the final organism.

Due to the natural host restriction of the canarypox virus and its attenuation as a vaccine strain, ALVAC is not pathogenic for canaries, other birds or mammals. Canarypox virus is genetically stable, replicates in the cell cytoplasm of the permissive host and its large genome allows the insertion of several genes. Among several potential insertion sites, two of them located in the inverted terminal repeats are commonly used. Generation of a recombinant canarypox virus starts with the cloning of the foreign gene of interest into a donor plasmid containing an expression cassette consisting of the promoter, the restriction endonuclease sites for transgene insertion and the flanking regions of the insertion locus [43]. The early/late vaccinia virus H6 promoter has been extensively used. Insertion of the cassette in the vector backbone is done by homologous *in vitro* recombina-

tion using chicken embryo fibroblasts infected with ALVAC and transfected with the donor plasmid. Due to the relatively high recombination rate, recombinant viral plaques may be screened directly by DNA hybridization or detection of the expressed foreign protein.

The genotypic and phenotypic traits of the final organism, its genetic stability, its tissue and host tropism and its safety for the target species and the environment must be documented for each new construct. The genetic stability of each ALVAC vaccine is indirectly confirmed by serial passages at a low multiplicity of infection in chicken embryo fibroblasts and immunoplaque assay or hybridization with a DNA probe specific for the inserted gene. The purity of the plaque virus is ensured by the absence of hybridization with a DNA probe specific for the deleted gene. The number of passages encompasses the passages from Master Seed virus to production batch. It is generally accepted that at least 95% of the plaques must express the transgene at the production batch level.

The host tropism of each new recombinant canarypox virus is tested both *in vitro* and *in vivo*. Serial passages are performed on primary cells and cell lines of the target species. Attempts to passage and adapt recombinant canarypox viruses on feline, canine or equine primary cells or cell lines of the same species have failed whatever the tested construct. Initial studies with ALVAC-Rabies in cells of human origin demonstrated that the block in viral replication occurred before DNA replication [44]. Micro-array profiling of cells infected with canarypox vector has recently confirmed that only early genes are transcribed in different types of human primary cells (Parrington, personal communication). The exact molecular events responsible for this block in viral replication are not yet identified for canarypox. Moreover, differences have been reported between avipoxviruses on different cell types [45].

The absence of change in the tropism of ALVAC after insertion of a foreign gene is confirmed in the safety studies carried out in canary birds and other species. The safety of each new recombinant canarypox virus is tested in canary birds in comparison with the parental strain by inoculation with a high dose via the transcutaneous route. Whatever the transgene, the recombinant canarypox virus does not induce clinical signs apart from mild local lesions at the inoculation site. The virus can be isolated from the skin at the site of inoculation and from various organs, but titres progressively decrease over time until virus cannot be detected. All recombinant viruses tested so far have been at least as safe as the parental strain, and no change of tropism has been observed. Because domestic birds may be in close contact with vaccinated animals, safety of ALVAC based vaccines is also tested in chickens. Control of each new master seed virus includes a safety test in mice by the intra-cerebral route. The LD<sub>50</sub> of ALVAC has been shown to be at least 10,000 times lower than the Copenhagen vaccinia virus strain administered by intra-cerebral route in newborn and 3-week-old mice [46]. In addition, the inoculation of 10<sup>9</sup> TCID<sub>50</sub> virus in nude immun-

odeficient mice did not cause any lesions, or any signs. These data showed, indirectly, the absence of *in vivo* replication of ALVAC. The safety data in nude mice highlight that the lack of replication cannot be attributed to an active immune response of the host and therefore is an inherent property of the canarypox virus.

The risk of horizontal gene transfer or recombination must be evaluated for each new recombinant vaccine in the context of its use. Canarypox virus is used as a vector in species which are not a reservoir of avipoxvirus, reducing thereby the risk of *in vivo* recombination. Nonetheless, cats have occasionally been infected with cowpox virus [47] and although there has been no report of recombination between avipoxviruses and orthopoxviruses, this risk must be evaluated before the release of recombinant canarypox virus vaccines. The likelihood to have co-localization (infection of the same cells in the same host) of a recombinant canarypox virus and a cowpox virus in natural conditions is extremely low. In addition, the genetic distance between avipoxvirus and orthopoxvirus minimizes the risk of recombination. This was confirmed by BLAST analyses of the region of the insertion sites showing that the risk of horizontal transfer of the transgene is very low.

In the European Union, registration of vaccines using the recombinant DNA technology must be done through the centralized procedure managed by Committee for Veterinary Medicinal Products (CVMP) at the European Medicines Evaluation Agency (EMA). In the USA, it is regulated by Animal and Plant Health Inspection Service and Center for Veterinary Biologics (APHIS/CVB) of United States Department of Agriculture (USDA) in compliance with the National Environmental Policy Act. In addition to the general guidelines governing the development and registration of veterinary vaccines, specific guidelines apply for live recombinant vector vaccines with a strong emphasis on the safety for the target species and the environment. Authorization to do field trials is granted at the national level and requires a complete risk analysis (equivalent to part II.H in the European Union; SIF in the USA). In addition to the risk assessment, the applicant must explain how the risk will be managed in the trial. In the USA, the public is notified about the request to do field trials with the recombinant vaccine through the Federal Register. The overall "Finding Of Non Significant Impact" process may take from 1 to 2 years, and may add considerable delays to the overall development.

#### 4. ALVAC as a technology platform

Several ALVAC-based vaccines have now been developed and licensed for veterinary use. The acquired expertise on the development and manufacturing of recombinant canarypox virus vaccines helps for the rapid generation of optimized constructs. As soon as the sequence of the immunogen is known, synthetic genes can be made and inserted into the ALVAC genome. ALVAC constructs are easier to handle than dangerous pathogens like H5N1 avian influenza virus, West-

Nile virus or Nipah virus. This technology platform facilitates the rapid generation of new constructs, which may be an advantage in case of emerging diseases. At the industrial level, the same manufacturing process is used for the various ALVAC-vaccines ensuring batch-to-batch consistency.

#### 5. Future prospects

Improving the efficacy and safety of conventional vaccines, providing rapid solutions for emerging diseases, and addressing new targets like chronic infections and cancer will probably be the main driving force in the development of new recombinant canarypox virus vaccines for veterinary use.

As an example, a canarypox virus vector expressing feline interleukin-2 was tested for local immunomodulation in cats with fibrosarcoma [48]. In the absence of immunotherapy, tumour recurrence was found in 61% of animals within a 12-month period following treatment with surgery and iridium-based radiotherapy. In contrast, only 28% of cats receiving ALVAC-feline IL2 exhibited tumour recurrences. Several ALVAC-based vaccines expressing tumour-associated antigens are currently being evaluated in humans [49] and may offer opportunities for companion animals as well.

So far, canarypox-vectored vaccines have been licensed only for companion animals and horses. However, ALVAC has also a potential in livestock, especially for emerging diseases. A recent experiment demonstrated the efficacy of ALVAC expressing VP2 and VP5 genes of Blue Tongue Virus (BTV) 17 in preventing BTV-induced viremia in sheep [50]. In pigs, canarypox virus expressing either the envelope fusion protein (F) or glycoprotein (G) of Nipah virus (NiV) protected pigs against a virulent challenge and more importantly prevented nasal and pharyngeal NiV shedding [51].

Advances in molecular biology, immunology, bio-informatics and vaccine technology have led to a better understanding of innate and specific immunity, a more detailed characterization of the pathogens and their interaction with the host and the development of new antigen expression platforms, more defined adjuvants and needle-free administration devices. Other technology platforms like DNA vaccines are emerging [5]. Combination of different expression platforms in a prime-boost setting is very promising, especially in chronic infections requiring strong CMI [52,53].

#### 6. Conclusion

Registration of any vaccine is based on a benefit/risk analysis. For live recombinant vectored vaccines, the risks for the target animal and the environment (including humans) are an important concern, and must be thoroughly evaluated in compliance with regulatory guidelines. Risk assessment requires a number of specific *in viro* and *in vivo* studies,

which are submitted to and reviewed by the regulatory authorities. Although live recombinant vectored vaccines are highly regulated, there is no major hurdle in the registration of such vaccines provided the risk is scientifically assessed and managed.

Consequently, live recombinant vectored vaccines are usually better characterized than classical attenuated vaccines, and their safety more documented.

As a non-replicative vector, ALVAC offers an attractive benefit/risk ratio, which in combination with its convenience of use, explains its success in the market place. The ongoing trials in various species including humans, suggest that other applications will be developed in the near future.

## References

- [1] Chang YF, Appel MJ, Jacobson RH, Shin SJ, Harpending P, Straubinger R, et al. Recombinant OspA protects dogs against infection and disease caused by *Borrelia burgdorferi*. *Infect Immun* 1995;63(9):3543–9.
- [2] Marciani DJ, Kensil CR, Beltz GA, Hung CH, Cronier J, Aubert A. Genetically-engineered subunit vaccine against feline leukaemia virus: protective immune response in cats. *Vaccine* 1991;9(2):89–96.
- [3] Rau H, Revets H, Balmelli C, McCullough KC, Summerfield A. Immunological properties of recombinant classical swine fever virus NS3 protein in vitro and in vivo. *Vet Res* 2006;37(1):155–68.
- [4] van Drunen Little, van den Hark S. Rationale and perspectives on the success of vaccination against bovine herpesvirus-1. *Vet Microbiol* 2006;113(3–4):275–82.
- [5] Lorenzen N, LaPara SE. DNA vaccines for aquacultured fish. *Rev Sci Tech Off Int Epiz* 2005;24(1):201–13.
- [6] Blancou J, Kieny MP, Lathé R, Lecocq JP, Pastoret PP, Soutébot JP, et al. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. *Nature* 1986;322(6077):373–5.
- [7] Brochier B, Blancou J, Thomas I, Languet B, Artois M, Kieny MP, et al. Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. *J Wildl Dis* 1989;25(4):540–7.
- [8] Mackowiak M, Maki J, Motes-Kreimeyer L, Harbin T, Van Kampen K. Vaccination of wildlife against rabies: successful use of a vectored vaccine obtained by recombinant technology. *Adv Vet Med* 1999;41:571–83.
- [9] Taylor J, Weinberg R, Kawaoka Y, Webster RG, Paoletti E. Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine* 1988;6(6):504–8.
- [10] Taylor J, Trimarchi C, Weinberg R, Languet B, Guillemin F, Desmettre P, et al. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 1991;9(3):190–3.
- [11] Taylor J, Weinberg R, Languet B, Desmettre P, Paoletti E. Recombinant fowlpox virus inducing protective immunity in non-avian species. *Vaccine* 1988;6(6):497–503.
- [12] Huang Z, Elankumaran S, Yunus AS, Samal SK. A recombinant Newcastle disease virus (NDV) expressing VP2 protein of infectious bursal disease virus (IBDV) protects against NDV and IBDV. *J Virol* 2004;78(18):10054–63.
- [13] Fischer L, Tronel JP, Pardo-David C, Tanner P, Colombet G, Minke J, et al. Vaccination of puppies born to immune dams with a canine adenovirus-based vaccine protects against a canine distemper virus challenge. *Vaccine* 2002;20(29–30):3485–97.
- [14] Dartel R, Bublot M, Laplace E, Bouquet JF, Audonnet JC, Riviere M. Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. *Virology* 1995;211(2):481–90.
- [15] Mellencamp MW, Long MTL, Gibbs PEG, Seino KSS, Beachboard SEB, Zhang S. Safety and efficacy of a live West-Nile virus chimera vaccine using a model of induced West-Nile virus clinical disease. In: Abstracts from International Veterinary Vaccines and Diagnostics Conference, 2006. p. 139.
- [16] Webster DP, Dunachie S, McConkey S, Poulton I, Moore AC, Walther M, et al. Safety of recombinant fowlpox strain FP9 and modified vaccinia virus Ankara vaccines against liver-stage *P. falciparum* malaria in non-immune volunteers. *Vaccine* 2006;24(15):3026–34.
- [17] Barouch DH, Nabel GJ. Adenovirus vector-based vaccines for human immunodeficiency virus type 1. *Hum Gene Ther* 2005;16(2):149–56.
- [18] Petri S, Greer CE, Thudium K, Due B, Legg H, Liu H, et al. An alphavirus replicon particle chimera derived from venezuelan equine encephalitis and sindbis viruses is a potent gene-based vaccine delivery vector. *J Virol* 2003;77(19):10394–403.
- [19] Schleiss MR, Bernstein DI, Passo M, Parker S, Meric C, Verdier F, et al. Lack of induction of autoantibody responses following immunization with cytomegalovirus (CMV) glycoprotein B (gB) in healthy CMV-seronegative subjects. *Vaccine* 2004;22(5):687–92.
- [20] Johnson DC, McFarland EJ, Muresan P, Fenton T, McNamara J, Read JS, et al. Safety and immunogenicity of an HIV-1 recombinant canarypox vaccine in newborns and infants of HIV-1-infected women. *J Infect Dis* 2005;192(12):2129–33.
- [21] Levy Y, Durier C, Lascaux AS, Meiffredy V, Gahery-Segard H, Goujard C, et al. Sustained control of viremia following therapeutic immunization in chronically HIV-1-infected individuals. *AIDS* 2006;20(3):405–13.
- [22] Karanikas V, Lurquin C, Colau D, van Baren N, De Smet C, Lethe B, et al. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant canarypox virus. *J Immunol* 2003;171(9):4898–904.
- [23] Ullenhag GJ, Frodin JE, Mosolits S, Kiaii S, Hassan M, Bonnet MC, et al. Immunization of colorectal carcinoma patients with a recombinant canarypox virus expressing the tumor antigen Ep-CAM/KSA (ALVAC-KSA) and granulocyte macrophage colony-stimulating factor induced a tumor-specific cellular immune response. *Clin Cancer Res* 2003;9(7):2447–56.
- [24] van Baren N, Bonnet MC, Dreño B, Khammari A, Dorval T, Piperno-Neumann S, et al. Tumor and immunologic response after vaccination of melanoma patients with an ALVAC virus encoding MAGE antigens recognized by T cells. *J Clin Oncol* 2005;23(35):9008–21.
- [25] Spaner DE, Astsaturov I, Vogel T, Petrella T, Elias I, Burdett-Radoux S, et al. Enhanced viral and tumor immunity with intranodal injection of canarypox viruses expressing the melanoma antigen, gp100. *Cancer* 2006;106(4):890–9.
- [26] Nacs J, Radaelli A, Edghill-Smith Y, Venzon D, Tsai WP, Morghen C de G, et al. Avipox-based simian immunodeficiency virus (SIV) vaccines elicit a high frequency of SIV-specific CD4+ and CD8+ T-cell responses in vaccinia-experienced SIVmac251-infected macaques. *Vaccine* 2004;22(5–6):597–606.
- [27] Paillet R, Kydd JH, Sindle T, Hannant D, Edmund Toulemonde C, Audonnet JC, et al. Antibody and IFN-gamma responses induced by a recombinant canarypox vaccine and challenge infection with equine influenza virus. *Vet Immunol Immunopathol* 2006;112(3–4):225–33.
- [28] Tartaglia J, Jarrett O, Neill JC, Desmettre P, Paoletti E. Protection of cats against feline leukemia virus by vaccination with a canarypox virus recombinant, ALVAC-FL. *J Virol* 1993;67:2370–5.
- [29] Poulet H, Brunet S, Boularand C, Guiot AL, Leroy V, Tartaglia J, et al. Efficacy of a canarypox virus-vectored vaccine against feline leukaemia. *Vet Rec* 2003;153:141–5.
- [30] Hofmann-Lehmann R, Tandon R, Boretto FS, Meli ML, Willi B, Cattori V, et al. Reassessment of feline leukaemia virus (FeLV) vaccines with novel sensitive molecular assays. *Vaccine* 2006;24(8):1087–94.
- [31] Flynn JN, Dunham SP, Watson V, Jarrett O. Longitudinal analysis of feline leukemia virus-specific cytotoxic T lymphocytes: correlation with recovery from infection. *J Virol* 2002;76:2306–15.

- [32] El Garch H, Richard S, Piras F, Leard T, Poulet H, Andréoni C, et al. Feline leukemia virus (FeLV) specific IFN $\gamma$ + T-cell responses are induced in cats following transdermal vaccination with recombinant FeLV vaccine. *Int J Appl Res Vet Med* 2006;4(2):100–8.
- [33] Backer RJ. Feline fibrosarcomas in vaccination sites. *Feline Pract* 1998;26:18–20.
- [34] Siger L, Bowen RA, Karaca K, Murray MJ, Gordy PW, Loosmore SM, et al. Assessment of the efficacy of a single dose of a recombinant vaccine against West Nile virus in response to natural challenge with West Nile virus-infected mosquitoes in horses. *Am J Vet Res* 2004;65(11):1459–62.
- [35] Edlund Toulemonde C, Daly J, Sindle T, Guigal PM, Audonnet JC, Minke JM. Efficacy of a recombinant equine influenza vaccine against challenge with an American lineage H3N8 influenza virus responsible for the 2003 outbreak in the United Kingdom. *Vet Rec* 2005;156(12):367–71.
- [36] Pardo MC, Bauman JE, Mackowiak M. Protection of dogs against canine distemper by vaccination with a Canarypox virus recombinant expressing canine distemper virus fusion and hemagglutinin glycoproteins. *Am J Vet Res* 1997;58(8):833–6.
- [37] Haase CJ, Hagerty TL, Larson LJ, Schultz RD. Advantages of using a recombinant canarypox vectored canine Distemper virus vaccine instead of modified live canine Distemper vaccine. In: Conference of Research Workers in Animal Disease. 2006.
- [38] Grosenbaugh DA, Backus CS, Karaca K, Minke JM, Nordgren RM. The anamnestic serologic response to vaccination with a canarypox virus-vectored recombinant West Nile virus (WNV) vaccine in horses previously vaccinated with an inactivated WNV vaccine. *Vet Ther* 2004;5(4):251–7.
- [39] Grosenbaugh DA, Leard T, Pardo MC. Protection from challenge following administration of a canarypox virus-vectored recombinant feline leukemia virus vaccine in cats previously vaccinated with a killed virus vaccine. *J Am Vet Med Assoc* 2006;228(5):726–7.
- [40] Minke JM, Siger L, Karaca K, Austgen L, Gordy P, Bowen R, et al. Recombinant canarypoxvirus vaccine carrying the prME genes of West Nile virus protects horses against a West Nile virus-mosquito challenge. *Arch Virol Suppl* 2004;18:221–30.
- [41] De Bruyn G, Rossini AJ, Chiu YL, Holman D, Elizaga ML, Frey SE, et al. Safety profile of recombinant canarypox HIV vaccines. *Vaccine* 2004;22(5–6):704–13.
- [42] Durchfeld B, Baumgartner W, Herbst W, Brahm R. Vaccine-associated canine distemper infection in a litter of African hunting dogs (*Lycodon pictus*). *Zentralbl Veterinarmed B* 1990;37(3):203–12.
- [43] Moss B. Genetically engineered poxviruses for recombinant gene expression, vaccination and safety. *Proc Natl Acad Sci USA* 1996;93:11341–8.
- [44] Taylor J, Meignier B, Tartaglia J, Languet B, VanderHoeven J, Franchini G, et al. Biological and immunogenic properties of a canarypox-rabies recombinant, ALVAC-RG (vCP65) in non-avian species. *Vaccine* 1995;13(6):539–49.
- [45] Well SC, Nilssen O, Traavik T. Avipoxvirus multiplication in a mammalian cell line. *Virus Res* 2005;109(1):39–49.
- [46] Tartaglia J, Perkus ME, Taylor J, Norton EK, Audonnet JC, Cox WI, et al. NYVAC: a highly attenuated strain of vaccinia virus. *Virology* 1992;188:217–32.
- [47] Chantrey J, Meyer R, Baxby D, Begon M, Bown KJ, Hazel SM, et al. Cowpox: reservoir hosts and geographic range. *Epidemiol Infect* 1999;122(3):455–60.
- [48] Jourdiere TM, Moste C, Bonnet MC, Delisle F, Tafani JP, Devauchelle P, et al. Local immunotherapy of spontaneous feline fibrosarcomas using recombinant poxviruses expressing interleukin 2 (IL2). *Gene Ther* 2003;10(26):2126–32.
- [49] Moingeon P. Recombinant cancer vaccines based on viral vectors. *Dev Biol (Basel)* 2004;116:117–22.
- [50] Boone JD, Balasuriya UB, Karaca K, Audonnet JC, Yao J, He L, et al. Recombinant canarypox virus vaccine co-expressing genes encoding the VP2 and VP5 outer capsid proteins of bluetongue virus induces high level protection in sheep. *Vaccine* 2007;25(4):672–8.
- [51] Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, Roth JA, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol* 2006;80(16):7929–38.
- [52] Telfer MC, Pu R, Pollock D, Vitsky A, Tartaglia J, Paoletti E, et al. Efficacy evaluation of prime-boost protocol: canarypoxvirus-based feline immunodeficiency virus (FIV) vaccine and inactivated FIV-infected cell vaccine against heterologous FIV challenge in cats. *AIDS* 1998;12(1):11–8.
- [53] Rogers WO, Baird JK, Kumar A, Tine JA, Weiss W, Aguiar JC, et al. Multistage multiantigen heterologous prime boost vaccine for *Plasmodium knowlesi* malaria provides partial protection in rhesus macaques. *Infect Immun* 2001;69(9):5565–72.
- [54] Stephensen CB, Welter J, Thaker SR, Taylor J, Tartaglia J, Paoletti E. Canine distemper virus (CDV) infection of ferrets as a model for testing Morbillivirus vaccine strategies: NYVAC- and ALVAC-based CDV recombinants protect against symptomatic infection. *J Virol* 1997;71(2):1506–13.
- [55] Plotkin SA, Cadoz M, Meignier B, Meric C, Leroy O, Excler JL, et al. The safety and use of canarypox vectored vaccines. *Dev Biol Stand* 1995;84:165–70.
- [56] Menon AG, Kuppen PJ, van der Burg SH, Offringa R, Bonnet MC, Harinck BI, et al. Safety of intravenous administration of a canarypox virus encoding the human wild-type p53 gene in colorectal cancer patients. *Cancer Gene Ther* 2003;10(7):509–17.
- [57] Triozzi PL, Strong TV, Bucy RP, Allen KO, Carlisle RR, Moore SE, et al. Intratumoral administration of a recombinant canarypox virus expressing interleukin 12 in patients with metastatic melanoma. *Hum Gene Ther* 2005;16(1):91–100.